

The Oral Microbiome in Dental Caries

IZABELA STRUŻYCKA

Department of Comprehensive Care, Medical University of Warsaw, Poland

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Abstract

Dental caries is one of the most common chronic and multifactorial diseases affecting the human population. The appearance of a caries lesion is determined by the coexistence of three main factors: acidogenic and acidophilic microorganisms, carbohydrates derived from the diet, and host factors. Socio-economic and behavioral factors also play an important role in the etiology of the disease. Caries develops as a result of an ecological imbalance in the stable oral microbiom. Oral microorganisms form dental plaque on the surfaces of teeth, which is the cause of the caries process, and shows features of the classic biofilm. Biofilm formation appears to be influenced by large scale changes in protein expression over time and under genetic control. Cariogenic microorganisms produce lactic, formic, acetic and propionic acids, which are a product of carbohydrate metabolism. Their presence causes a decrease in pH level below 5.5, resulting in demineralization of enamel hydroxyapatite crystals and proteolytic breakdown of the structure of tooth hard tissues. *Streptococcus mutans*, other streptococci of the so-called non-mutans streptococci group, *Actinomyces* and *Lactobacillus* play a key role in this process. Dental biofilm is a dynamic, constantly active metabolically structure. The alternating processes of decrease and increase of biofilm pH occur, which are followed by the respective processes of de- and remineralisation of the tooth surface. In healthy conditions, these processes are in balance and no permanent damage to the tooth enamel surface occurs.

Key words: cariogenic bacteria, dental caries, dental biofilm, oral microbiome

Introduction

Dental caries is one of the most common chronic infectious diseases worldwide and endangers humans throughout their life, not only during childhood or adolescence. It is the most common cause of tooth loss and pain in the oral cavity (Featherstone, 2004; Edelstein, 2006).

Epidemiological studies indicate a differentiated incidence of caries in various countries. In developed countries, a decreasing prevalence of the disease is observed. The reason for improvement of oral health conditions is attributed to diverse factors, including water fluoridation, use of fluoride toothpaste, a healthier diet containing sucrose substitutes and oral health education. (Konig, 2004; Marthaler, 2004). In developing countries, the incidence of dental caries still remains at a high level, and Poland unfortunately belongs to this group of nations (Robert and Sheiham, 2002; Wierzbicka *et al.*, 2012).

Dental caries, as a process determined by lifestyle, may be subject to activation in each period of human life if hygiene and diet are neglected even for a period as short as a few weeks (Nyvad and Fejerskov, 1997;

ten Cate, 2001). In adverse conditions even the most resistant teeth will be affected by this disease (Kidd and Fejerskov, 2004). In the early stages the caries progress, can be stopped or reversed, but if left untreated, the disease may cause dysfunctions of the masticatory apparatus and systemic odontogenic infections.

The oral cavity as a complex ecosystem

The oral cavity is an extremely diverse, dynamic and unique ecosystem in the human body with a characteristic feature being the instability of its ecological conditions (Marsh, 2005). The oral cavity consists of a mucous membrane covered with a keratinized stratified squamous epithelium (*e.g.* the palate) and a non-keratinized epithelium, the papillary surface of the tongue and hard non-shedding structures of the teeth above and below the gingival margin, with different surfaces, grooves and hollows. These sites constitute separate ecological niches promoting the development of microorganisms, with each niche having a distinctive microbiome (Dewhirst *et al.*, 2010; Frandsen *et al.*, 1991; Paster *et al.*, 2006;).

* Corresponding author: I. Strużycka, Zakład Stomatologii Zintegrowanej, Warszawski Uniwersytet Medyczny, Miodowa 18, 00-246 Warszawa; e-mail: istruzycka@gmail.com

The environment of the oral cavity is constantly transformed with age, and thus the oral microbiome changes as well. During the first two months of a baby's life, bacteria colonize only mucosal surfaces, and with the eruption of the deciduous teeth, the non-shedding hard surfaces appear, which are also colonized by microorganisms. The changing factors such as emergence of the milk and permanent teeth, tooth extractions, caries cavities, fillings, dentures and sometimes toothlessness may affect the ecosystem of the oral microbiome (Tweman *et al.*, 2000).

Subsequent tooth eruptions cause at the same time the emergence of a new environment – a gingival sulcus with a separate source of nutrients (Abusleme *et al.*, 2013). Environmental conditions such as temperature, salinity, access to oxygen, access to nutrients, pH conditions and the redox potential have an impact on the ecosystem and contribute to the species composition of biofilms present at each location (Takahashi and Nyvad, 2011). Transient changes in the stability of the oral microbiome may be also caused by diet, variability of the salivary flow, or long-term use of antibiotics (Sheiham, 2001; Nasidze *et al.*, 2009).

In addition, the entire oral cavity is constantly moistened with saliva, a rich secretion containing countless amounts of different types of biologically active ingredients. Saliva is used by the oral biofilm as a delivery system. It is the major source of nutrients for microorganisms, peptides, carbohydrates and the content of these substances largely determines the quality and quantity of microorganisms inhabiting the oral cavity (Nasidze *et al.*, 2009). Saliva has antibacterial, antiviral and antifungal properties. It includes proteins such as lysozyme (causing bacteriolysis), lactoferrin (inhibiting the bacterial growth), lactoperoxidase (blocking the glucose metabolism), statherin and other protein components of saliva, e.g. mucins, immunoglobulin A, G and M and a number of other glycoproteins (Hannig *et al.*, 2005; van Nieuw Amerongen *et al.*, 2004). Saliva also contains proline-rich glycoproteins (PRPs), which act as receptors allowing for strong attachment of bacteria to the acquired pellicle, which covers the tooth surface (Marsh, 2005).

The flow of saliva allows admission of nutrients essential for bacteria, but on the other hand, it promotes the mechanical removal of bacteria from the colonized surfaces. The flow of saliva, the mastication process and oral hygiene remove a large number of bacteria from the oral cavity. Therefore, the flow rate of saliva and its quality are critical not only in the initiation and development of dental caries but also in the process of remineralization of early carious lesions (Garcia-Godoy and Hics, 2008). An increased salivary secretion rate helps to reduce the time of teeth enamel exposure to acids and accelerates normalization of pH in their environ-

ment after a meal containing cariogenic food products. Thus, it facilitates maintenance of the microbial plaque pH at a level that ensures the integrity of the mineral composition of the enamel. Maintaining an ionic balance between the enamel and saliva is possible due to the presence of the buffering compounds – carbohydrates and phosphates (Hics *et al.*, 2003).

Transmission of cariogenic microorganisms

As the toothless oral cavity of the fetus is sterile, a human first becomes colonized by a normal flora at the moment of passage through the birth canal (Berkowitz, 2006; Caufield and Griffen, 2000; Cephas *et al.*, 2011). During birth, the first days of life and feeding by the mother, certain microorganisms colonize the oral cavity, and the pre-dentate infant's mucosal surfaces are the only suitable sites for colonization in the mouth. In research study performed by Cephas *et al.*, (2011), high bacterial diversity was noted in saliva of adults and infants. *Streptococcus* was the predominant genus in infant saliva. *Veillonella*, *Neisseria*, *Rothia*, *Haemophilus*, *Gemella*, *Granulicatella*, *Leptotrichia* and *Fusobacterium* were also predominant genera in infant samples, while *Haemophilus*, *Neisseria*, *Veillonella*, *Fusobacterium*, *Oribacterium*, *Rothia*, *Treponema* and *Actinomyces* were predominant in adults.

The colonization of children by microorganisms considered to be associated with the development of caries is related to their transfer by saliva from people in the child's closest environment. Most data show that oral colonization by *S. mutans* occurs through direct and indirect contact with related persons whose oral cavity is colonized by such microorganisms (Berkowitz, 2006; Caufield *et al.*, 1993; Mattos *et al.*, 2001).

Studies with the use of molecular methods indicate that the vertical transmission of these bacteria from mother to child is the main route for their early acquisition in the oral cavity (Berkowitz, 2003; Caufield and Griffen, 2000; Redmo-Emanuelsson and Thornqvist, 2001). Bacteria enter the child's oral cavity from their caregivers, and usually the mother is the first transmitter (Caufield and Griffen, 2000; Milgrom *et al.*, 2000). The transmission of cariogenic bacteria occurs in approximately 60% of infants when the level of microorganisms in the mother's saliva amounts to 10^5 or more colony forming units per millilitre of saliva (CFU/ml) compared to 6% when the bacterial level in the mother's saliva is 10^3 CFU/ml of saliva (Berkowitz, 2006). The intrafamilial transmission of cariogenic microorganisms is different in populations of various cultures. There are also reports describing microbial genotypes found in children that are different from the isolates derived from their mothers or other family

members, which indicates the existence of additional sources and horizontal pathways of cariogenic bacteria transmission (Mattos *et al.*, 2001).

Etiopathogenesis of dental caries

Dental caries is often described as the physical and chemical processes of demineralization and remineralization occurring on the tooth surface. Yet the essence of this disease is more complex. Theories about the etiology of dental caries are still evolving in tandem with the development of molecular biology and introduction of improved microscopy research techniques (Fejerskov, 2004; He and Shi, 2009; Nyvad *et al.*, 2013; Wood *et al.*, 2000). Dental caries is a multifactorial disease. A variety of factors, including microbial, genetic, immunological, behavioral and environmental interact to contribute to dental caries onset and development. (Aas *et al.*, 2008; Selwitz *et al.*, 2007). Diet is one of the most important factors associated with colonization of the oral cavity by cariogenic bacteria (van Loveren, 2001).

Recent advances including data from the Human Microbiome Project have led to a new paradigm for understanding chronic bacterially mediated diseases. Dental caries occurs as a result of a shift in the composition of a biofilm community specific to the human tooth surface. Frequent carbohydrate intake can disrupt the ecology of this community by the selection of acidogenic and acid tolerant species, these acidogenic communities are responsible for caries development (Nasidze *et al.*, 2009).

The cariogenic properties of microorganisms in the oral cavity are associated with their ability to live and grow on a hard and non-shedding tooth surface, the level of colonization of dental plaque, increasing in relation to consumption of sucrose, the ability of rapid processing of monosaccharides to acids (acid formation), the ability to survive in conditions of low pH (acidophilicity), the production of extracellular polysaccharides (EPS), which facilitate adhesion to the tooth surface and building of a matrix, and the production of intracellular polysaccharides (IPS) (Horiuchi *et al.*, 2009; Selwitz *et al.*, 2007). The cariogenic potential of microorganisms is directly related to the consumption of carbohydrates, in particular of sucrose, and this fact has been proved long time ago by laboratory tests carried out on gnotobiotic animals (Fitzgerald and Keyes, 1960).

A caries lesion develops on a specific surface of the tooth, under the mature dental biofilm coating it for a long period. It is now believed that the disease is caused by microorganisms belonging to the natural flora of the oral cavity (Peterson *et al.*, 2013; Scheie and Petersen, 2004; ten Cate, 2006). It is suggested that in

the oral microbiome, there is a dynamic equilibrium between microorganisms as well as between the microflora and the host, and the disease develops as a result of a microbiological imbalance within the biofilm (Hoiby *et al.*, 2011; Hojo *et al.*, 2009; Marsh, 2012).

Such a situation occurs when conditions in the local environment on the tooth surface change. In the case of dental caries, the change may include repeated high concentrations of sugar resulting in a decrease in plaque pH due to acidophilic and acidogenic microorganisms. In conditions of low pH, cariogenic microorganisms multiply efficiently and take over the dominant position in the biofilm. They produce weak acids, which are a product of carbohydrate metabolism (lactic, formic, acetic and propionic acids). The presence of these acids results in a decrease in pH below the critical value (5.0–5.5), leading to demineralization of hydroxyapatite crystals in the tooth enamel (Garcia-Godoy and Hics, 2008; Hics *et al.*, 2003).

The mechanism of the disease is the same in all types of dental caries and is associated with the loss of minerals (demineralization) and proteolytic breakdown of the structure of the tooth hard tissues under the influence of the attack of acids produced by bacteria (Kidd and Fejerskov, 2004; Marsh, 2012; Selwitz *et al.*, 2007). The escape of calcium phosphates and carbonates from the enamel surface usually leads to the creation of a caries cavity in the enamel. The demineralization process is sometimes reversible in the early stages and then remineralization occurs with the capture of calcium, phosphate and fluoride. Important features of the carious process are the episodes of demineralization followed by periods of remineralization, *i.e.* repair and recrystallization, when parts of the lost compounds are re-built. Remineralization is the tooth's natural repair mechanism consisting of diffusion of minerals from saliva to the porous demineralized tooth surface (Marsh *et al.*, 2011). Fluoride administered in order to accelerate this process, works as a catalyst for the incorporation process of calcium and phosphate into the crystalline structure of the hydroxyapatite tooth enamel (Marinho *et al.*, 2003).

The origin, progress or inhibition of dental caries are determined by the equilibrium status between protective factors, most of them being components of saliva (Ca^{2+} , phosphates, fluoride, protective proteins of the pellicle, saliva antibacterial components and external factors), and pathological factors (cariogenic bacteria, a dysfunction of the salivary glands, frequent consumption of carbohydrates). A preponderance of pathological factors results in the processes of demineralization and dental caries (Kidd and Fejerskov, 2004; Garcia-Godoy and Hics, 2008).

In most people, the demineralization and remineralization processes occur on the tooth surface many times

during the day. Remineralization occurs as a result of pH adjustment in the oral cavity with saliva acting as a buffer. If plaque remains on the tooth surface for an extended period of time, as a result of bacteria activity, an irreversible caries lesion appears under this biofilm, which is a symptom of advanced disease (Featherstone, 2004). Very early stages of dental caries in the enamel are not detectable by simple clinical or radiological methods.

The supragingival microbiome in dental caries

Opinions on the composition of microflora responsible for initiation of the carious process change in line with new research on this topic. Previous hypotheses point to the primary role of *Streptococcus mutans* as the primary pathogen in the etiology of dental caries in children and adults. (Aas *et al.*, 2008; Hoiby *et al.*, 2011; Tanzer *et al.*, 2001).

Recent advances in high throughput genomics now allow for a comprehensive survey of bacterial species present in the oral cavity. 16S rRNA gene analysis have made it possible to comprehensively examine the composition of microbial communities and to study differences between health and disease (Belda *et al.*, 2012; Filoche *et al.*, 2010; Peterson *et al.*, 2013).

Recently published papers offer a partly different perspective on the role of bacteria in the caries process, referred to as the so-called extended caries ecological hypothesis, trying to prove that the role of *S. mutans* in the initiation of dental caries may not be as dominant as it was previously assumed, but there is an agreement that they have the highest cariogenic potential (Takashi and Nyvad, 2008; He and Shi, 2009). Van Ruyven *et al.*, (2000) have proposed a model for succession in caries that takes into account carbohydrate intake, “low pH groups of bacteria” and mutans streptococci and lactobacilli. According to the group of researchers who adhere to this theory, the initiation of the caries process results from activity of many aciduric and acidogenic microorganisms, known in literature as the non-mutans streptococci and *Actinomyces* (McLean *et al.*, 2012).

Colonization of the cleaned tooth surfaces proceeds involving *Streptococcus sanguinis*, *Streptococcus oralis* and *Streptococcus mitis*, belonging to the “non-mutans streptococci”. These three species constitute as much as 95% of all *Streptococci* present in dental plaque, and 56% of the total initial colonizers of the tooth surface. At the initial stage of plaque development, *S. mutans* represents only 2% of the other streptococci population. (Horiuchi *et al.*, 2009).

The microflora colonizing the clinically healthy enamel surface contains mainly non-mutans streptococci and *Actinomyces*, and acidification of the plaque

environment is mild and rare. There is an equilibrium maintained between the demineralization and remineralization processes, with a predominance of the latter (Bik *et al.*, 2010). In the oral cavity under conditions with a modified physical environment (e.g. through poor hygiene, frequent delivery of sugar, or too low a flow of saliva that buffers pH), decreases in pH follow after each meal, which entails a growing acidification of the plaque environment, and consequently changes the composition of the bacterial flora (Takahashi and Nyvad, 2011; van Ruyven *et al.*, 2000). This leads to a selective multiplication in dental plaque of bacteria known as low-pH non-mutans streptococci, which are more acidogenic and acidophilic in comparison to the original non-mutans strains, which further shifts the equilibrium of the de- and remineralization processes towards demineralization, leading to increased development of *S. mutans* and *Lactobacillus* and progression of the carious process (Garcia-Godoy and Hics, 2008; Nasidze *et al.*, 2009; Takashi and Nyvad, 2008).

In conditions of severe and long-term acidification of the plaque environment (even pH < 4,0), the most acidogenic and acidophilic bacteria start to dominate: *Streptococcus mutans* and *Streptococcus sobrinus*, *Lactobacilli*, the most acidophilic strains of non-mutans streptococci, *Actinomyces*, *Bifidobacteria* and yeast. The carious process progresses with changes in the composition of the bacterial plaque (Aas *et al.*, 2008; Horiuchi *et al.*, 2009).

The percentage of *S. mutans* increases in the dental plaque in the stage of white-spot enamel lesions, but non-mutans streptococci still remain dominant. The proportion of *S. mutans* amounts to about 30% of the total plaque microflora in the advanced stages of caries (Horiuchi *et al.*, 2009). In addition to the “non-mutans” streptococci, *Actinomyces* are also detected in the initial stages of plaque development.

Moreover, research also indicates that other microorganisms present in dental plaque that have been considered to be non-cariogenic up to now, such as *Bifidobacterium*, *Propionibacterium*, *Streptococcus salivarius*, *Streptococcus oralis*, *Streptococcus milleri*, *Enterococcus faecalis*, *Actinomyces naeslundii* and *Actinomyces viscosus*, may also result in dental caries (Aas *et al.*, 2008).

Dental plaque as a biofilm

Application of modern research techniques, has allowed fuller understanding of many important phenomena occurring on the tooth surface near the gingival margin and in the gingival sulcus, the site of bacterial dental calculus deposits. This techniques has also contributed to the knowledge of the highly complex structure of dental plaque. Dental plaque was one of

the first structures formed by bacteria that has been described as a biofilm (Marsh, 2005; ten Cate, 2006; Hoiby *et al.*, 2011). Over 700 bacterial species have been isolated from the human oral cavity and the majority of them are associated with dental biofilm. Many years of research have shown that this biofilm is a highly specialized, co-ordinated, multi-species form of microorganism life, permanently located on the tooth surface in a matrix, surrounded by a layer of extracellular polysaccharides (EPS). It is created by the layered growth of microorganisms existing as separated microcolonies, mainly bacteria capable of adhering to each other, which can form a community where spatially distributed populations can interact. When organized in biofilms, the oral micro-organisms are less susceptible to *antimicrobials* and more resistant to immunological defense systems (Davies, 2003; Dufour *et al.*, 2013; Stoodley *et al.*, 2002).

Microorganisms in the oral cavity form two types of biofilm on the surface of the tooth: the supra-gingival plaque and the sub-gingival plaque, which differ significantly in the composition of the bacterial flora. Supragingival plaque is dominated by Gram positive bacteria, including *Streptococcus mutans*, *Streptococcus salivarius* *Streptococcus mitis* and *Lactobacillus*, while subgingival plaque is dominated by Gram-negative anaerobic bacteria, such as *Actinobacillus*, *Campylobacter* spp., *Fusobacterium nucleatum*, *Porphyromonas gingivalis* (Hojo *et al.*, 2009; He and Shi, 2009; Marsh, 2012). The cause of dental caries is usually the supra-gingival microbiome. The sub-gingival microbiome is associated with gingivitis and periodontal disease (Abusleme *et al.*, 2013).

The formation and development of biofilm takes place in three main steps: attachment of the initial pioneer species, which leads to an increase in the biofilm mass due to colonization, co-adhesion, co-aggregation of other species of microorganisms, production of extracellular polysaccharides and separation of bacteria from the surface of the biofilm and their spread in the environment of the oral cavity. (Hoiby *et al.*, 2011; Scheie and Petersen, 2004; ten Cate, 2006).

Since microorganisms are not able to colonize the cleaned tooth surfaces, which are deprived of any external components, the presence of the acquired pellicle is necessary for adhesion. The acquired pellicle is formed on the tooth surface immediately upon their brushing or professional cleaning treatments. This process is estimated to take minutes. It consists of saliva and proteins from gingival fluid adsorbed on to the cleaned surface. Gingival fluid is composed of different host-derived molecules rich in proline, tyrosine, histidine, including proteins and agglutinins which act as a source of receptors that are recognized by various oral bacteria, mucins and other glycoproteins. The initial stage of bac-

terial adhesion to the tooth surface includes interaction of the superficial substances of microorganism with the components of saliva contained in the acquired pellicle. As the attached bacteria cells grow and divide, many will start to express a biofilm phenotype, which will include the secretion of extracellular polymeric substances (EPS) with polypeptides, carbohydrates and nucleic acids (Hojo, 2009; Kolebrander *et al.*, 2010).

In the initial non-specific phase of biofilm formation, when bacteria that form it are located at a considerable distance apart, the electrostatic, hydrophobic and van der Waals forces allow for reversible adhesion of microorganisms (Hics *et al.*, 2003; Scheie and Petersen, 2004). This initial phase of adhesion becomes irreversible later due to a specific reaction between bacteria adhesins and PRPs (proline-rich glycoproteins) on the surface of the acquired pellicle. Accumulation of biofilm microorganisms is very fast as a result of the interspecies aggregation of streptococci with actinomyces, as well as agglutination of microorganisms within one species, which leads to aggregation of new bacterial species with the already settled organisms. Among the early colonizers of the biofilm, different species of *Streptococcus* spp., *Eikenella* spp., *Actinomyces* spp., *Haemophilus* spp., *Prevotella* spp., *Capnocytophaga* spp., *Priopionibacterium* spp., and *Veillonella* spp. have been identified in 60–90% of cases. The later colonizers include *Fusobacterium nucleatum*, *Actinobacillus* spp., *Prevotella* spp., *Eubacterium* spp., *Treponema* spp. and *Porphyromonas* spp. (Kolebrander *et al.*, 2010; Marsh, 2012; Nasidze *et al.*, 2009).

Several salivary components have been shown to have a role in microbial adhesion to the pellicle, for instance, salivary oligosaccharide-containing glycoproteins may serve as receptors for oral streptococci and the salivary proline-rich protein 1 and statherin have been implicated as receptors for type 1 fimbriae of *Actinomyces viscosus* (Gibbons *et al.*, 1988; Marsh, 2005; Scheie and Petersen, 2004). The wide range of streptococcal species produce the most well-studied oral adhesins, including antigen I/II, PaG, SspA, amylase-binding proteins, and type 1 fimbriae-associated protein. Adhesins produced by other oral bacteria have also been identified. Bacterial forms colonize the pellicle acquired during the day, which is transformed into dental biofilm.

A major role in the formation of biofilm is attributed to extracellular polysaccharides (EPS: Exopolysaccharides) containing, among others, mannose and glycosidic residues, which form a bacterial capsule or are released into the environment, where they become part of mucus. The composition of EPS varies depending on the bacterial strain and environmental conditions. Consist of exopolisaccharides, proteins and extracellular DNA. The biofilm matrix is increased by further

precipitation of salivary glycoproteins, mainly through the creation of extracellular disaccharides. A particularly important role in the creation of plaque mass and adhesion of bacteria is attributed to polysaccharide polymers-soluble ones such as glucan and fructan, and insoluble ones, such as mutan (Hojo *et al.*, 2009; Marsh, 2011). The cariogenic streptococci form extracellular polymers in the enzymatic reactions involving α -glucosidases. For example, *S. mutans* produces two glucosidases. One leads to the formation of insoluble glucan with α -1,3-glucan-binding called mutan. The other it creates a bond α -1,6 characteristic of the soluble glucan known as dextran. Dextran acts as a substrate for further metabolism in the event of shortage of saccharides in food. Sticky mutan is involved in increasing of the plaque mass and enhances adhesion of the cariogenic microorganisms. From fructose, with involvement of β -fructosidase, a polymer called fructan or levan is derived. Fructan, like dextran, constitutes an extracellular metabolic substrate. *S. mutans* produces a rare soluble fructan called inulin (Biswas *et al.*, 2005; Takashi and Nyvad, 2008).

Extracellular polysaccharides are created also by other bacteria in the oral cavity, such as *S. sanguis* and *A. viscosus*, but a major role in their production is attributed to *S. mutans*. One of the important functions of EPS, in addition to the significant role in the processes described above, is the protection of these microorganisms against the host defense system and this is especially meaningful for the pathogenic nature of cariogenic microorganisms. The coating made of polysaccharides, due to its hydrophilic nature, effectively protects bacteria from phagocytosis (Allison, 2003).

After the adhesion phase, the process of building of the biofilm structure begins with microbial multiplication and differentiation (Belda *et al.*, 2012, Filoche *et al.*, 2010). Immediately after adhesion of bacteria to the surface or to other bacteria, activation or inhibition of specific gene expression occurs, with biofilm maturation being associated with changes in activity of particular genes in relation to the environmental conditions. Changes in gene expression involve the occurrence of relevant phenotypic traits. For example, due to changes in the environment such as limited access to oxygen, the metabolism of cells changes: the activity of anaerobic metabolic pathways of glycolysis increases, and the synthesis of certain enzymes is inhibited (Ling *et al.*, 2010; Roberts and Mullany, 2010). Production of flagellin, the protein included in the flagella of Gram-negative bacteria, is inhibited. As a result, certain structures disappear, *e.g.* flagella, which are no longer needed in this "stationary" form of existence within the biofilm (Marsh, 2005).

Microorganisms in biofilm are arranged in orderly, complex structures that are highly differentiated, con-

sisting of bacterial microcolonies. The mature biofilm, coated with a layer of exopolysaccharides, on the surface of the tooth is sometimes composed of a countless number of microcolonies, separated from one another by numerous fluid filled channels, which creates a unique communication system of biofilm cells. The fluid circulating in the channels flows past microcolonies, delivering nutrients, oxygen, enzymes, metabolites, signal molecules and removing waste products (Wood *et al.*, 2000; Stoodley *et al.*, 2002). In addition, it acts as a protection for its inhabitants against external factors including antibiotics, antibodies, bacteriophages, leukocytes. Thus it plays a triple role: surrounds, fastens and protects. The mutual proximity of the cells encourages exchange of genetic information by transfer of plasmids, including those encoding for resistance to antimicrobial substances and antibiotics (Davies, 2003; Roberts and Mullany, 2010).

Biological interactions have a role in structuring communities. They can occur within a community, across communities between microbiota and host, and between microbiota and their preators (Biswas *et al.*, 2005; Kolebrander *et al.*, 2012; Kubonika *et al.*, 2012).

A very important property of bacterial biofilm, much better developed than in the case of planktonic cells, is the ability to communicate with each other and regulate metabolic processes. Quorum sensing (QS) signaling represents a signaling pathway that is activated as a response to cell density. The stimuli of QS systems are signal molecules called autoinducers, their concentration is a function of microbial density. It depends on the cell population density in a particular site. Bacteria have complex gene expression programs which are up-regulated when they co-habit in dense populations. For example, their activity is changed and slow down their metabolism which is essential to prevent an accumulation of metabolic waste or the depletion of nutrients (Irie and Parsek, 2008; Kolebrander *et al.*, 2002; Scheie and Petersen, 2004). Bacteria are able to accurately identify the chemical nature of the signals and their threshold concentration in the environment, which allows for their specific growth and control of physiological and metabolic processes of the entire population. Streptococci have evolved various means of coping with the deleterious effects of environmental stressors and avoiding the host immune system. Recently several studies have shown that streptococci colonizing the mouth and upper respiratory tract are able to mount complex stress responses in order to persist and successfully survive competition in their ecological niche. Using a small quorum sensing peptide pheromone acting as a stress-inducible 'alarmone', oral streptococci synchronize the gene expression of a specific group of cells to coordinate important biological activities. (Dufour *et al.*, 2013). Bacteria, thanks to the

chemical signals they send, are able to control important life processes such as bioluminescence, production of virulence factors, symbiosis, conjugation, mobility, spore formation, colony growth and biofilm formation (Kolebrander *et al.*, 2002; Marsh, 2005; Spoering and Gilmore, 2006). Gram-positive and Gram-negative bacteria have developed completely different QS transmission systems for signaling molecules. Functions of auto-inducers in Gram-negative bacteria are fulfilled by acyl homoserine lactones (AHLs), and in Gram-positive by specific oligopeptides and auto inducers-2 (AI-2). AHL diffuses into cytoplasm and up-regulates the transcription of specific genes thus influencing the production of vital proteins. In *S. mutans*, QS is mediated by a competence-stimulating peptide (CSP). It was shown that when CSP reached a threshold level, a subpopulation of the bacteria lysed. By doing so they provided DNA to the environment which was then taken up by other competent bacteria, which is essential for exchange of genetic material and evolutionary process. These specific signaling molecules are used for communication between cells of the same or different bacterial populations or strains. Bacteriocins might also function as QS signals and have a direct effect on biofilm composition (Irie and Parsek, 2008; Li *et al.*, 2002; Miller and Bassler, 2001; Spoering *et al.*, 2006).

The formation of the layer of microorganisms is a dynamic process. Adhesion, growth, removal and re-attachment constitute a continuous process and thus dental plaque microbiom undergoes a constant reorganization. This naturally-constructed biofilm consortia of bacteria may reach a thickness of 300–500 cells on the surface of the teeth. The surface of mature biofilm may release individual cells, which can then form a biofilm on other sites, teeth, tooth surfaces, gingival sulcus or transform into the planktonic form. In this manner biofilm constitutes a subsisting source, continuously sowing bacteria into the body fluids and to the environment.

Bacteria living in the biofilm form usually do not give up to the defense mechanisms activated by the immune system, and antibiotic action is also limited (Hojo *et al.*, 2009; Roberts and Mullany, 2010). Only occasionally do antibiotics or other antimicrobial measures manage to penetrate the sticky polysaccharide substance surrounding the biofilm. The variety of environmental conditions and the diversity of bacteria species in biofilms provide a sufficient protection against antimicrobial agents (Marsh, 2012). Concentrations of antimicrobial agents used to destroy the biofilm components must be very high, even up to several hundred times greater than necessary for destruction of the free-living bacteria (Roberts and Mullany, 2010; Scheie and Petersen, 2004). The best method to fight dental biofilm is to prevent colonization of the tooth surface, which

constitutes a potential base for biofilm formation, and thereby inhibit its development. However, it is essential to comply with the generally accepted principles of dental caries prophylaxis.

Summary

Dental caries is a multifactorial disease. The caries lesion present on the tooth surface may be active or arrested and reflects the activity in the biofilm covering tooth surface. Dental biofilm bacteria function as a highly organized and integrated microbial community. They cooperate and compete by different mechanisms, resulting in change of biofilm structure and function. Microbial composition of dental biofilm depends on the complex host-microbial and microbial-microbial interactions. Some of them are better understood. Knowledge on the mechanisms of genetic regulation involved in oral microbiome formation is still insufficient.

A better understanding of processes of oral biofilm formation and function is necessary to the development of novel more successful, more rational approach for antimicrobial strategies oral disease prevention and treatment.

A very important point is education and instructing patients on oral hygiene and the need for mechanical means of plaque removal, with the simplest method for the patient being the brushing of teeth. Local antibacterial agents may be used as a supplement and not a substitute for professional and home oral hygiene.

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